EVIDENCE ON DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF SODIUM NITRITE

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Outline of Presentation

- **Background: chemical properties, metabolism, and exposure**
- **General toxicity**
- **Reproductive toxicity**
- **Developmental toxicity, non-cancer endpoints**
- **Transplacental carcinogenesis**
- **Summary comments**

Sodium nitrite, or nitrous acid sodium salt

- CAS No. 7632-00-0
- molecular formula: $NaNO_2$
- molecular weight: 69.00
- water soluble, inorganic salt

NO_3^-
nitrate

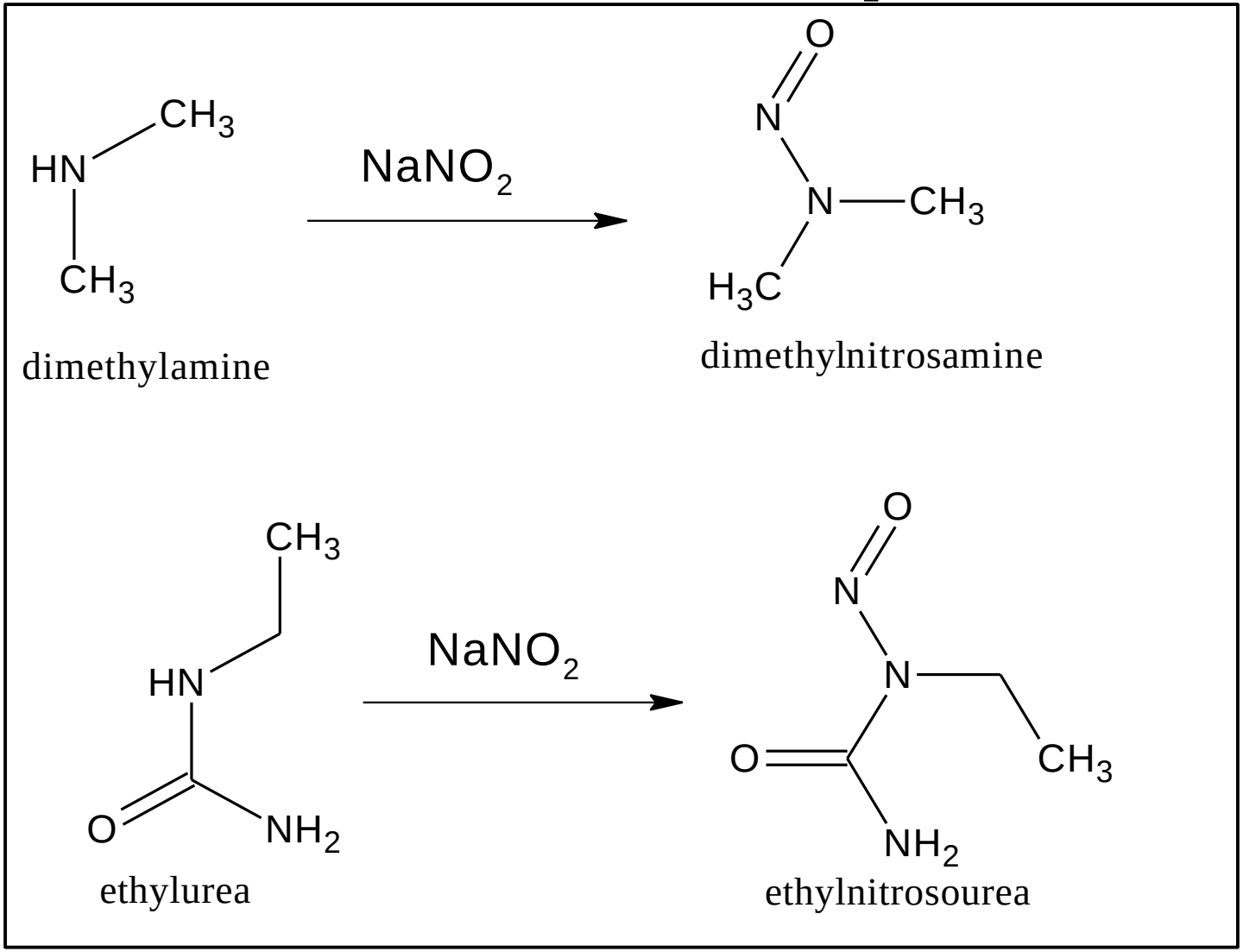
NO_2^-
nitrite

- About 5% of an ingested dose of nitrate is reduced to nitrite by microorganisms resident in the oral cavity

- Can also be reduced in the stomach, if pH is sufficiently high

OEHHA

Formation of N-nitroso Compounds



Exposure

Direct exposure to sodium nitrite or nitrite ion, or metabolic conversion from nitrate

- Sodium nitrite added to cured meats as a preservative, color fixative, and flavor enhancer
- Nitrite and nitrate naturally present in vegetables
- Nitrite and nitrate can be present in drinking water

Methemoglobin (MetHb)

- Nitrite in the blood participates in an oxidation reaction with hemoglobin (Hb) to form MetHb
- MetHb cannot carry oxygen
- Ascorbic acid (and other antioxidants) may slow the rate of MetHb formation
- Reduction of MetHb to Hb is catalyzed by NADPH-MetHb-reductase
- Methylene blue is used therapeutically to accelerate MetHb-reductase activity

Effects on Fertility

- **No human data**

- **Six pair-based animal studies of sodium nitrite, and one of potassium nitrite**
  - NTP continuous breeding study in mice
  - One-generation feeding study in rats
  - One-generation study of rats fed sodium nitrite-treated meat
  - Three-generation drinking water study in rats
  - One-generation drinking water study in mice
  - Subchronic study of rats fed sodium nitrite-treated fish
  - Drinking water study of potassium nitrite in guinea pigs

- **No effects observed on parameters of fertility**

Effects on Other Reproductive Parameters

- **Female Reproductive Toxicity**
  - Five studies reported on non-fertility reproductive endpoints in females
  - One report of reduced offspring growth suggested a possible lactation-suppressing effect in mice
  - Inflamed reproductive organs and placental degeneration in guinea pigs given potassium nitrite during pregnancy

- **Male Reproductive Toxicity**
  - Five studies reported on non-fertility reproductive endpoints in males
  - Two studies reported on testicular changes in rats, which could not be conclusively attributed to sodium nitrite exposure

New Data Relevant to Reproductive Toxicity (NTP Draft Technical Report; Peer Review Date, 18 May 2000)

- **14- week drinking water studies in male and female rats and mice**
  - Significantly reduced sperm motility in both species at higher concentrations; apparent concentration response
  - Testicular degeneration in mice at the two highest concentrations
  - Estrous cycle length significantly increased at higher concentrations in mice
  - Survival not affected; reduced body weights and increased relative weights for some organs at the highest concentrations
  - Rats showed more clinical symptoms of methemoglobinemia than did mice; no NOAEL for increased methemoglobin in rats

- **2-year drinking water studies in male and female rats and mice**
  - No notable histopathological changes in the reproductive organs of male or female rats or mice
  - Survival not affected; reduced body weights at highest concentration

Effects on Development – Human Data

- **No data available on sodium nitrite**

- **Case-control studies of two populations exposed to nitrate in drinking water during pregnancy**
  - Australia: significant increased risk for CNS malformations, also for all malformations with an apparent dose effect
  - Canada: non-significant increased risk for CNS malformations

- **Study of pregnant women given nitrosatable drugs**
  - Can undergo nitrosation to form N-nitroso compounds
  - Significantly increased risk for major malformations with exposure during first four months of pregnancy

Effects on Development in Guinea Pigs

- **Sodium nitrite (s.c.)**
  - Two studies; several small experiments
  - Ascorbic acid deficient vs non-deficient animals
  - Increased abortion in treated/deficient animals at lower doses than in treated/non-deficient animals
  - Prevented by methylene blue
  - Fetal deaths associated with increased maternal MetHb

- **Potassium nitrite (drinking water)**
  - One study; small groups of guinea pigs
  - Males and females exposed
  - One maternal death, reduced weight gain at high dose
  - All fetuses died at two highest doses
  - Increased fetal loss in all treated litters, no statistics

Effects on Development in Mice

- **Study of erythropoiesis on gestation days 14, 16, and 18**
  - Treatment of dams by gavage, throughout gestation
  - Maternal effects not reported
  - No effects on viability or gross morphology
  - Significant changes in parameters of hepatic erythropoiesis

- **Developmental toxicity study**
  - Treatment of dams by gavage, gestation days 6-15
  - Minimal maternal toxicity at highest doses
  - Apparent decrease in viability probably not related to treatment

- **Drinking water study**
  - Treatment of dams via drinking water, gestation days 7-18
  - No significant effects on dams or fetuses

Effects on Development in Rats

- **Three studies of neurobehavioral parameters**
  - Treated drinking water to dams from gestation day 13
  - Some animals given nimodipine (neuroprotective, antihypoxic)
  - Impairments of discrimination learning and long-term retention of passive avoidance
  - Effects on open field activity
  - Hyperreactivity to footshock, and prolonged stress response
  - Effects on ingrowth of nerve fibers
  - Effects prevented or alleviated by nimodipine

- **One drinking water study; exposure throughout gestation and lactation**
  - Maternal effects not reported
  - Birthweights similar among groups
  - Mean litter size lower in treated animals; no statistics

Placental Transport

- **Nitrite found in fetal blood following maternal dosing (oral or injection) of rats and guinea pigs**
- **Nitrite shown to cross the placenta in dairy cows**
- **Nitrite dosing of pregnant animals resulted in elevated methemoglobin in fetuses as well as mothers**

Toxicosis

- **Methemoglobinemia in pregnant and lactating animals**
  - Pregnant rats more sensitive to acute lethality
  - Chronically exposed pregnant rats became severely anemic, while non-pregnant rats maintained control Hb levels
  - *In vitro*, erythrocytes from pregnant mice had higher velocity of MetHb formation

- **Maternal and fetal toxicosis in infused dairy cows**
  - Maternal effects: increased MetHb, reduced blood pressure, increased heart rate, and decreased arterial PO_2
  - Nitrite appeared in fetal circulation, and fetal MetHb levels rose
  - Fetal heart rates were affected, and fetal PO_2 depressed
  - No increase in frequency of premature delivery or abortion

Transplacental carcinogenesis

- Animal studies and other relevant data
- Epidemiologic studies

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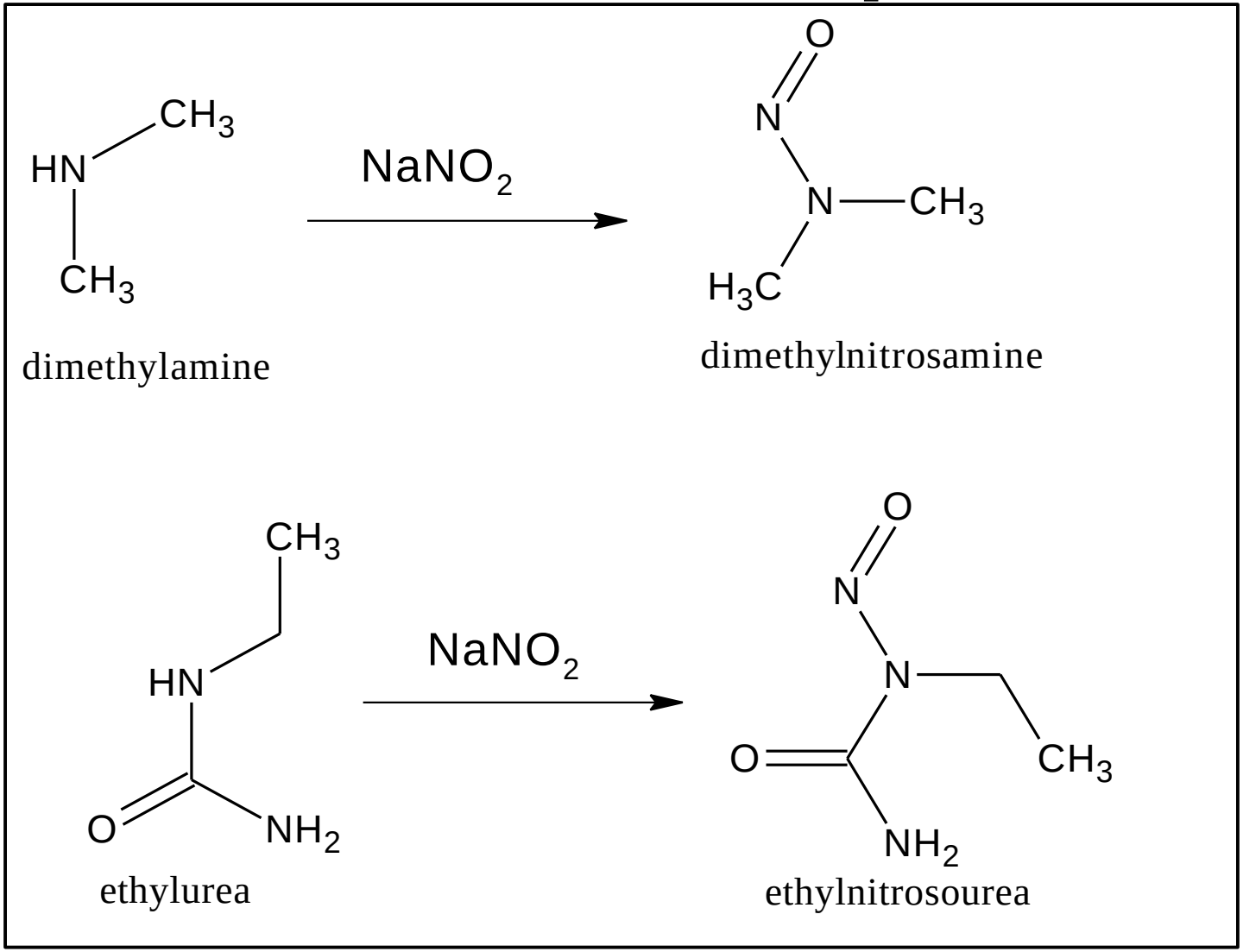

Sources of

- endogenous production
  - e.g., histamine, spermine, tyramine
- foods
  - e.g., meats, fruits, vegetables, grains
- beverages
  - e.g., wine, beer, coffee, tea
- medicines
  - e.g., antihistamines, antibiotics, tranquilizers

Inhibitors of nitroso f

- Vitamin C (ascorbic acid)
- Polyphenols
- Other antioxidants (e.g., vitamin E)

Endogenous production of vitamin C

Human	No
Guinea pig	No
Hamster	Yes
Rat	Yes
Mouse	Yes

Other Relevant Data: Cancer studies of N-nitroso compounds

- Nitrosamines
  - tumors of the liver, esophagus, nasal and oral mucosa, kidney, pancreas, bladder, lung, thyroid
- Nitrosoureas
  - tumors of lymphatic and nervous system

Other Relevant Data: Transplacental cancer studies of N-nitroso compounds

- Nitrosoureas
  - brain, spinal cord, PNS tumors in the rat
  - hematopoetic tumors in the mouse
  - kidney, liver, lung tumors in the hamster

Animal studies of transplacental carcinogenesis

Species	**$NaNO_2$ alone**	**$NaNO_2$ + amine/amide**
Hamster	2	3: ethylurea (2), morpholine
Rat	3	3: L-citrulline, morpholine, diethylamine
Mouse	2	1: cimetidine

Prenatal exposure

Hamster (gd 15)	$NaNO_2$ alone +ethylurea	--- PNS tumors (*inhibited by ascorbate)	Rustia, 1975
Hamster (gd 12-15)	$NaNO_2$ + ethylurea	PNS tumors	Rustia & Schenken, 1976
Rat (gd 13-23)	$NaNO_2$ alone + citrulline	--- Wilms' tumor (kidney)	Ivankovic, 1979

Pre- & postnatal exposure

Hamster	$NaNO_2$ alone + morpholine	--- ---	Shank & Newberne, 1976
Rat	$NaNO_2$ alone + morpholine	Lymphoreticular, total tumors (other than liver & angiosarcomas) Liver carcinoma, Angiosarcoma of Liver, Lung	Shank & Newberne, 1976
Rat	$NaNO_2$ alone +diethylamine	--- ---	Druckery *et al*., 1963
Mouse	$NaNO_2$ alone + cimetidine	Lung, Lymphoma Lung	Anderson *et al*., 1985
Mouse	$NaNO_2$ alone	---	Hawkes *et al*., 1992

Epidemiologic studies

- Childhood leukemia
- Childhood brain tumors

Epidemiologic approach

- Dietary intake during pregnancy as recalled by mothers
- Exposure: frequency of consumption of cured meats
- Controls matched on date of birth, race; identified by random digit dialing
- Adjustment in analyses for other factors (e.g., SES, other exposures, maternal age)

Childhood Leukemia and Maternal Diet During Pregnancy

Study characteristics

- Three case-control studies
- Two smaller studies, one large (232 cases)
- Various ages (<1 year, 0-14, 1-10)
- Leukemia types mixed or separated
- Largest study had detailed exposure information

Childhood Leukemia and Maternal Diet During Pregnancy

Maternal consumption of cured meats

- No statistically significant increased risks
- Possible dose-response effect observed for hot dogs in large study ($p \leq 0.1$)
- Apparent difference in risk by leukemia type in study of infants

Childhood Leukemia: Other Relevant Data

Childhood consumption of cured meats

- Largest study:
  Dose-related trend for hot dogs ($p \leq 0.001$)
  Significant OR=5.8 in high dose group
- Infant study: No data
- Study of one leukemia type (ALL):
  Significant risk associated with hot dog consumption in those who took no vitamins

Childhood Brain Tumors and Maternal Diet During Pregnancy

Study characteristics

- Nine case-control studies of brain tumors
- Published in 1982 - 1996
- U.S. and Canada, France and Australia
- Cases identified through cancer registries
- Age at diagnosis: 0 - 14 or 15 years (6 studies); 0-6 (2 studies), 0-19 (1 study)
- Largest study had 540 cases (801 controls)

Childhood Brain Tumors and Maternal Diet During Pregnancy

<u>All cured meats</u>

- Statistically significant increased risks in four studies & one published abstract
- ORs ranging from 1.4 to 2.5
- Dose-response effect observed in largest study

Childhood Brain Tumors and Maternal Diet During Pregnancy

<u>Hot dog consumption</u>

- Statistically significant increased risks in four of six studies
- ORs ranging from 1.4 to 2.3
- Possible dose-response effect observed in one large study reported only in abstract form

Childhood Brain Tumors: Maternal Vitamin Use

- Prenatal vitamin use decreased risk when used throughout pregnancy
  OR=0.54, $p<0.05$
- Prenatal vitamin use reduced risk associated with cured meat consumption
  Median intake of cured meat
  OR=2.4, $p<0.05$ without vitamins
  OR=1.3 $p=0.05$ with vitamins

Childhood Brain Tumors: Other Relevant Data

- Childhood consumption of cured meats: four studies with mixed results
- Seven of 10 published case-control studies of adult brain tumor risk found an association with cured meat intake

Additional Considerations

- Potential for biased recall of diet
- Association with food group could be proxy for another exposure (e.g., fat)
- Sodium nitrite exposure levels not quantified
- Effect examined in a variety of populations
- Relatively consistent effect across a range of studies of childhood brain tumors

Available Evidence

- **Reproductive toxicity**
  - No human data
  - Fertility in animals
  - Other reproductive endpoints in animals

- **Developmental toxicity (non-cancer endpoints)**
  - Human data on nitrates and nitrosatable drugs
  - Animal data from three species
  - Studies on placental transport, methemoglobinemia during pregnancy, and nitrite toxicosis in pregnant animals

- **Transplacental carcinogenicity**
  - Animal models
  - Human data on nitrite-containing foods